The Toxicity of Deglycosylated Ricin A Chain-containing Immunotoxins in Patients with Non-Hodgkin’s Lymphoma Is Exacerbated by Prior Radiotherapy: A Retrospective Analysis of Patients in Five Clinical Trials

John Schindler, Edward Sausville, Richard Messmann, Jonathan W. Uhr, and Ellen S. Vitetta

The Cancer Immunobiology Center and the Department of Microbiology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390 [J. S., J. W. U., E. S. V.], and the Developmental Therapeutics Program, National Cancer Institute, Bethesda, Maryland [E. S., R. M.]

ABSTRACT

A retrospective analysis of 102 patients with relapsed, non-Hodgkin’s lymphoma treated with two different ricin A chain-containing immunotoxins (ITs) in five Phase I clinical trials indicates that the dose-limiting toxicity, vascular leak syndrome, was more frequent and more severe in patients who had undergone prior radiotherapy (RT). Excluding patients with prior RT from the calculations of the maximum tolerated dose indicates that the maximum tolerated doses of these ITs had not been reached in any trial and are clearly higher than reported previously. Excluding patients with prior RT from future clinical trials may increase the dose of ITs that can be given in the absence of severe vascular leak syndrome.

INTRODUCTION

ITs\(^3\) are targeted toxins that use a targeting moiety such as a MAb or antibody-derived protein sequence coupled to a cytotoxic moiety, such as RTA (1). ITs that are designed to treat cancer have two potential advantages over conventional chemotherapy and radiotherapy: (a) they are targeted to the tumor cells, hence reducing toxicity to normal cells; and (b) they kill cells by mechanisms that are different from those mediated by standard cytotoxic agents and hence should kill chemorefractory cells. One disadvantage of ITs is their large size, which makes penetration into large tumors inefficient (2). Thus, ITs should perform most effectively in the setting of adjuvant therapy to treat MRD.

We have been developing two ITs to treat patients with B-cell tumors and particularly NHL. One IT is directed against the CD22 antigen and contains the murine IgG1 MAb, RFB4 (3). The other IT is directed against the CD19 antigen and contains the murine IgG1 MAb, HD37 (4). The CD22 and CD19 antigens are expressed on tumor cells in 60–70% and >90% of patients with NHL, respectively (5, 6). Both MAbs are conjugated to dgRTA. dgA does not bind to liver cells, thus avoiding hepatotoxicity and increasing serum half-life (7). The DLT of these RTA-containing ITs is VLS, which is manifested by decreased serum albumin, edema, and weight gain. The clinical spectrum of VLS-related side effects ranges from mild edema (requiring only supportive care) to respiratory failure (requiring ventilator support).

In Phase I clinical trials, both ITs have produced a number of either partial or complete responses in patients with relapsed or refractory lymphoma. The next step in the clinical evaluation of these agents is to use them in combination with conventional chemotherapy and/or RT to treat patients with MRD.

To develop regimens for combining dgRTA ITs with other conventional agents, SCID mice with human Daudi lymphoma cells have been used to optimize combination dose regimens (8). When ITs were combined with RT, toxicity was dependent on the temporal order of administration of the two agents (8). Hence, if the IT was administered prior to RT, SCID mice experienced no observed toxicity and had excellent tumor regressions (8). However, if the IT was administered after RT, SCID mice experienced weight loss and death. Necropsies of mice documented pulmonary edema (8).

In our last Phase I clinical trial in which a 50:50 mixture of the RFB4-dgA and HD37-dgA ITs (Combotox) was administered, we reported severe toxicity in patients who had prior RT (9). To explore a general association between IT toxicity and RT, we have now examined the data from our five Phase I trials. This analysis had two important goals: to determine (a) whether excluding patients with prior RT would reduce toxicity in patients entered into future trials; and (b) whether severe (grades 3 and 4) toxicity associated with prior RT in previous trials may have given us a misleadingly low MTD. Exclusion of patients with prior RT may therefore permit the administration of higher doses of ITs, leading to increased response rates and increased times to progression.
A retrospective analysis of 102 evaluable patients treated in five Phase I trials (9–12) revealed a statistically significant association between prior RT and severe toxicity. Indeed, the MTDs in four of the five studies had been defined only by the patients with prior RT; MTDs had not been reached in patients without a history of RT. In the three studies in which a comparison could be made, the MTDs for patients without prior RT was a minimum of one dose level higher than that for patients with prior RT.

**PATIENTS AND METHODS**

**Definition of MTD.** Two different ITs were used to treat >200 patients with NHL in five Phase I clinical trials (9–12). These trials used similar but not identical designs. The major objectives were to determine the MTDs as well as the pharmacokinetics and immunogenicity of these two ITs. In all cases, the MTD was defined as (a) the dose level at which one or more of three or fewer patients experienced grade 4 toxicity or three or more of six or fewer patients experienced grade 3 toxicity; or (b) the dose level below which there was a death on study attributable to the drug or the dose level below which a criterion in (a) was exceeded, e.g., two patients with grade 4 toxicity. Toxicities were graded according to the National Cancer Institute Toxicity Grading Scale (11), modified to include VLS, the most common DLT experienced with our ITs (9). Severe toxicity was defined as a toxicity of grade 3 or higher.

**Dose Regimens.** Two different dose regimens were used in these five clinical trials. The first regimen was a BI lasting 4 h. A course of treatment consisted of four BIs administered q.o.d. (total of 8 days). The second regimen was a CI administered over 8 days (9, 11). The two different ITs were administered individually by each regimen in the first four clinical trials. Both drugs were given together (Combotox) in a 1:1 ratio by the CI regimen in the fifth trial (9). MTDs were defined for one course of treatment.

**Data Analysis.** For the analyses in this report, patients were divided into those receiving prior RT (regardless of dosage, anatomic location of RT, or time since RT) and those without prior RT. Both groups had received one or more courses of chemotherapy prior to IT therapy. Each group was then evaluated to determine whether an MTD was defined for that group. To determine whether there was association between severe toxicity or death and prior radiation treatment, a two-tailed Fisher’s exact test was used.

**RESULTS**

Patients in all five trials had relapsed or refractory NHL, and most had bulky disease. Among the 102 evaluable patients treated in the five trials, 60 patients were males and 42 patients were females. The median age was 49 years (range, 20–80 years). There was no statistically significant difference in either the distribution of males and females or the distribution of ages between the patients with prior RT and the patients without prior RT. Table 1 summarizes the trial designs, numbers of patients, and the MTDs in each trial, and Table 2 summarizes the severe toxicities in the patients with and without prior RT.

By retrospective analysis, the MTDs in trials 1 (10), 2 (12), 3 (11), and 5 (9) were defined by patients with a history of RT.

To determine whether MTDs could be defined for each group within each trial, the patients were divided into those with and those without prior RT. As shown in Table 3 (row 1), in the first trial (10), one patient with prior RT experienced grade 4 toxicity at a total dose of 45 mg/m², and one patient in this group died at the 37 mg/m² dose level. The MTD was therefore the next lower dose (32 mg/m²). Two patients without prior RT received doses ≥37 mg/m² without exhibiting toxicity. Of 11 patients in this group, 2 patients experienced grade 3 toxicity at doses ranging from 5 to 52 mg/m² (1 at 19 mg/m² and 1 at 30 mg/m²). Additional patients would be required to define the MTD for this group.

As shown in Table 3 (row 2), in the second trial (12), the MTD for patients with prior RT was 16 mg/m². This MTD was defined by the two patients at the next dose level (24 mg/m²) who experienced grade 4 toxicity. For patients without prior RT, 24 mg/m² was tolerated with only one in five patients experiencing grade 3 toxicity. The MTD in these patients was therefore ≥24 mg/m².

As shown in Table 3 (row 3), in the third trial (11), the MTD for patients with prior RT was 19.2 mg/m²; seven of seven patients treated at 28.8 mg/m² experienced severe toxicity, including two deaths related to the IT. In contrast, only two of the first six patients (three of nine total) without prior RT experienced grade 3 toxicity at 28.8 mg/m². The dose was not escalated for these patients because the MTD for the study had been defined by the patients with prior RT. Thus, for patients without prior RT, the MTD was ≥28.8 mg/m².

As shown in Table 3 (row 4), in the fourth trial (12), there were too few patients in either group to define the MTD. There was a death on study attributable to toxic megacolon, which was thought to be unrelated to treatment. However, this patient had undergone RT to the pelvic area, and therefore, the megacolon could actually be related to therapy. Exclusion of this patient left one patient in the 9.6 mg/m² dose level (no toxicity reported) and one patient in the 19.6 mg/m² dose level (grade 3 toxicity) in the group of patients with prior RT. Thus, the MTD is undefined in this group. In the group without prior RT, two of five patients had grade 3 toxicity at 19.2 mg/m². Thus, the MTD was ≥19.2 mg/m².

As shown in Table 3 (row 5), in the fifth trial (Combotox; Ref. 9), in the group with prior RT there was a death on study at 10 mg/m². Therefore, the MTD for this group is <10 mg/m². In the group without prior radiation treatment, one of six patients treated at 20 mg/m² experienced grade 3 toxicity, and neither of two patients treated at 30 mg/m² experienced grade 3 toxicity. Thus, the MTD for this group is ≥20 mg/m².

### Table 1 Phase I clinical trials

<table>
<thead>
<tr>
<th>Study no. and IT</th>
<th>Schedule</th>
<th>No. of patients</th>
<th>MTD (mg/m²/course)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (RFB4-dgA; Ref. 10)</td>
<td>q.o.d. × 4, (BI)</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>2 (HD37-dgA; Ref. 12)</td>
<td>q.o.d. × 4, (BI)</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>3 (RFB4-dgA; Ref. 11)</td>
<td>CI for 8 days</td>
<td>22</td>
<td>19.2</td>
</tr>
<tr>
<td>4 (HD37-dgA; Ref. 12)</td>
<td>CI for 8 days</td>
<td>10</td>
<td>19.2</td>
</tr>
<tr>
<td>5 (Combotox; Ref. 9)</td>
<td>CI for 8 days</td>
<td>&lt;10 (no CTCs)</td>
<td>≥30 (+ CTCs)</td>
</tr>
</tbody>
</table>

*a CTC, circulating tumor cell.*
To determine whether there was a correlation between severe toxicity and prior RT, the patients from the five studies were divided into four groups. As shown in Table 4, there was a statistically significant association between severe toxicity and prior RT ($P = 0.004$). As shown in Table 5, there was also a statistically significant association between death on study and prior RT ($P = 0.011$).

Although severe toxicities in patients without RT were too infrequent to define an MTD in any trial, Table 6 lists the specific types of severe toxicities observed in patients with and without prior RT. The most common severe toxicity was VLS. Thus, VLS is not unique to patients with prior RT, but rather is increased in frequency and severity. Severe VLS was almost always associated with fluid accumulation in the lungs. Of the 12 patients who had RT and severe VLS, only 2 had a history of mediastinal radiation. Likewise, aphasia occurred in both groups and did not appear to be specifically radiation associated. However, prior RT increased the probability of severe VLS.

**DISCUSSION**

We evaluated two ITs in five Phase I clinical trials in 102 patients with NHL (9–12). Each IT was administered by a 4-h BI q.o.d. × 4 doses (8 days total) and by CI over 8 days. In the fifth trial, Combotox was administered by CI for 8 days. Each of

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the first four trials determined an MTD for a single IT using one dose regimen. The fifth trial determined the MTD of Combotox administered by CI. All trials demonstrated that the ITs had antitumor activity (9).

The key finding to emerge from this retrospective analysis is that IT-associated toxicity is more severe in patients who have had prior RT at any time, dose, or anatomical location. Indeed, if we exclude the data from these patients from our analyses, an MTD had not been reached for the individual ITs or for Combotox in any trial. Thus, patients with prior RT will be excluded from future trials. This is consistent with the ultimate goal of using ITs to treat patients with early disease and/or MRD. If these patients are excluded, higher doses should be well tolerated, and the response rates and duration of responses should increase accordingly. These findings argue for additional Phase I dose escalation trials that exclude patients with a history of RT. They also suggest that VLS might be less of a problem than we had suggested previously.

With regard to an explanation of why prior RT predisposed patients to more severe and more frequent IT-mediated VLS, several points can be made: (a) It has been reported that RT can cause long-lasting or permanent damage to the vasculature (13) and this could lead to greater sensitivity to IT-mediated VLS. (b) RTA ITs initiate VLS by binding to vascular endothelial cells (14), and an amino acid sequence in the RTA that appears to be responsible in binding has been identified (15, 16). If this sequence binds to vascular endothelial cells that are already damaged, the damage may be exacerbated. Alternatively, RT might cause one type of damage to the vasculature and IT may cause another type of damage. The two toxicities might be additive. (c) In our SCID xenograft model it appears that, unlike RT-mediated vascular damage, IT-mediated VLS is reversible (8). This may explain why when IT therapy is given first and RT is given 2 weeks later, the mice can tolerate the combined treatment.

The analysis of information derived from our five clinical trials argues for the exclusion of patients with a history of RT from treatment regimens containing these ITs. Furthermore, IT therapy in the setting of early disease or MRD will require an additional Phase I dose escalation trial to address the question of a recommended Phase II dose when patients with prior RT are excluded.

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REFERENCES

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